**Before the**
**FOOD AND DRUG ADMINISTRATION**
**Washington, D.C.** 6192 '04 JUN -3 P2:37

In re: Docket No. 2004P-0060

Filed on: June 3, 2004

**COMMENTS SUBMITTED BY WEIDER NUTRITION INTERNATIONAL, INC.**

Weider Nutrition International, Inc., ("Commenter") hereby submits the attached scientific report by Michael John Glade, Ph.D., CNS, FACN (Exhibit A) as responsive to the Petition of Rotta Pharmaceuticals, Inc. Dr. Glade's report responds to the Petition of Rotta Pharmaceuticals, Inc., concerning the pharmacological and pharmacokinetic properties of crystalline glucosamine sulfate. Commenter respectfully requests that FDA consider the attached report of Dr. Glade when evaluating the Petition filed by Rotta Pharmaceuticals, Inc.

Respectfully submitted,

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SUP 1

# EXHIBIT A

The central theme of the Rotta Pharaceuticals, Inc. petition filed February 13, 2004 (seeking FDA approval for certain glucosamine sulfate health claims) is that dietary supplementation with glucosamine sulfate reduces the risk of osteoarthritis, joint structure deterioration and related joint pain and limitation of function. With this we wholeheartedly agree; the weight of substantiating scientific evidence is massive.

However, another central theme of the document arises from the speculative, incorrect and unsubstantiated statements that "Other sources of glucosamine do not have the same quality, pharmacological, and pharmacokinetic properties of crystalline glucosamine sulfate" [pp. 2-3] and "These other forms of glucosamine (*i.e.*, glucosamine hydrochloride, N-acetyl-glucosamine, or other 'glucosamine sulfate' formulations) may not share the same quality, pharmacological, pharmacokinetic and, especially, clinical properties of crystalline glucosamine sulfate." [p. 46] No scientific evidence supports those statements. Despite reiteration of that theme throughout the petition, Rotta Pharmaceuticals, Inc. offers no proof demonstrating (or even evidence suggesting) that the physiological and biochemical mechanisms of action of Rotta's product at the cellular or tissue levels differ in any way from that of glucosamine HCl or any other non-Rotta glucosamine sulfate product.

In fact, the science which Rotta Pharmaceuticals, Inc. chose to ignore in its petition demonstrates quite conclusively that intact glucosamine sulfate *per se* has virtually no "bioavailability" and only exhibits biological activity in the prevention of human osteoarthritis *in* vivo after pre-absorption dissociation of the glucosamine and sulfate moieties (Setnikar *et al.*1984; Setnikar *et al.* 1986; Setnikar *et al.* 1993; Hoffer *et al.* 2001; Setnikar and Rovati 2001). Indeed, all of the available evidence indicates that glucosamine from any source is absorbed as either free glucosamine or glucosamine HCl, circulates as either free glucosamine HCl or bound to circulating proteins, and acts upon tissues and cells as either free glucosamine or glucosamine HCl (Setnikar *et al.*1984; Setnikar *et al.* 1986; Setnikar *et al.* 1993; Setnikar and Rovati 2001).

In rats and humans, ingested glucosamine sulfate is completely ionized to glucosamine and sulfate ions in the stomach (Setnikar *et al.* 1986). In studies in rats, 90% to 95% of the glucosamine from ingested glucosamine sulfate was absorbed intact (as free glucosamine and glucosamine HCl) into the blood (Setnikar *et al.*1984; Setnikar and Rovati 2001). About 30% of this newly absorbed free glucosamine and glucosamine HCl was incorporated into newly synthesized proteoglycans in articular cartilage tissues (Setnikar *et al.*1984; Setnikar and Rovati 2001). In studies in humans, consumption of 314 mg of crystalline glucosamine sulfate was followed by the absorption of about 280 mg (about 90%) of intact glucosamine and glucosamine HCl into the bloodstream; about 50% of this amount (about 140 mg) survived hepatic first-pass extraction intact (Setnikar and Rovati 2001). When the consumption of 1884 mg occurred as one bolus or in three divided intakes of 626 mg every 4 hours, there was no difference in total glucosamine and glucosamine HCl bioavailability to systemic tissues (about 40% to 50% of the amount of glucosamine ingested). Other investigators have reported that over 90% of the glucosamine ingested as glucosamine sulfate was absorbed intact into the human

enterohepatic circulation (Setnikar *et al.* 1986, Setnikar *et al.* 1993). One investigator reported that about 75% of ingested glucosamine sulfate was available to body tissues as free glucosamine and glucosamine HCl following hepatic first-pass extraction (Setnikar *et al.* 1993). All investigators have reported that glucosamine is absorbed in humans as free glucosamine or glucosamine HCl and not as glucosamine sulfate and that glucosamine circulates in humans as free glucosamine or free glucosamine HCl or bound to circulating proteins (no glucosamine sulfate has been detected in the circulation) (Setnikar *et al.*1984; Setnikar *et al.* 1986; Setnikar *et al.* 1993; Hoffer *et al.* 2001; Setnikar and Rovati 2001). In addition, even when glucosamine sulfate has been injected parenterally, only glucosamine HCl has been detected in the blood or urine of human volunteers (Setnikar and Rovati 2001, Setnikar *et al.* 1986, Setnikar *et al.* 1993). Based on their replicated findings, investigators have concluded that the available bioavailability data demonstrate that, in humans, glucosamine sulfate is a precursor of the biologically active substance, glucosamine (Setnikar *et al.* 1993).

Interestingly, in healthy subjects, the consumption of glucosamine sulfate was followed by increased serum sulfate concentration (Hoffer *et al.* 2001). In contrast, the consumption of sodium sulfate did not affect serum sulfate concentration, suggesting that dietary supplementation with glucosamine sulfate might facilitate dissociation of sulfate from glucosamine prior to absorption in the human gastrointestinal tract and thereby provide both glucosamine and free sulfate for proteoglycan synthesis.

Abundant published data demonstrate the misleading nature of the contention that only "crystalline glucoamine sulfate" is biologically active in the prevention of human osteoarthritis. For example, the addition of D-glucosamine to the culture medium nourishing rat chondrocyte cell cultures prevented interleukin-1β (IL-1β)-induced inhibition of the expression of UDP-glucuronosyltransferase I mRNA (Gouze *et al.* 2001; 2002) and of proteoglycan synthesis (Gouze *et al.* 2001; 2002), as well as IL-1β-induced activation of pro-apoptotic nuclear factor KB (NF-KB) (Gouze *et al.* 2002). The addition of glucosamine HCl to the culture medium of nonosteoarthritic equine articular cartilage explants in organ culture prevented IL-1β-induced increases in the activities of stromelysin-1, collagenase and gelatinase and bacterial lipopolysaccharide (LPS)- and IL-1β-induced increases in the production of NO and $PGE_2$ and the degradation of extracellular matrix proteoglycans (Fenton *et al.* 2000a; 2000b; 2002; Orth *et al.* 2002). Following transport across the chondrocyte cell membrane by the GLUT-2 and GLUT-4 glucose transporters (Dean *et al.* 1989; Pelletier *et al.* 1991), supplemental D-glucosamine stimulated the expression of IL-1 cell membrane receptor subtype II, which binds IL-1β with high affinity but produces an inactive receptor-ligand complex, effectively intercepting IL-1β-based signal transmission (Gouze *et al.* 2002).

Both glucosamine HCl and glucosamine sulfate added to the culture medium of nonosteoarthritic rat femoral articular cartilage explants in organ culture significantly increased the rates of collagen and proteoglycan synthesis and partially prevented nonsteroidal anti-inflammatory drug- (NSAID)-induced inhibition of proteoglycan synthesis (Karzel and Domenjoz 1971; Vidal y Plana *et al.* 1978). When D-glucosamine was added to the culture medium of nonosteoarthritic bovine articular cartilage explants

in organ culture in concentrations that significantly inhibited IL-1β-induced aggrecanase cleavage of aggrecan, lactate production was unaffected and D-glucosamine was incorporated into newly-synthesized chondroitin sulfates (Noyszewski *et al.* 2001). Glucosamine HCl also stimulated sulfate incorporation into chondroitin sulfates in the extracellular matrix of nonosteoarthritic bovine articular cartilage explants in organ culture (Roden 1956). In addition, when added to the culture media of chondrocytes harvested from osteoarthritic human articular cartilage, in which adhesion of chondrocytes to fibronectin and overall protein synthesis are significantly inhibited while extracellular collagenase activity is significantly increased, D-glucosamine restored the adhesive properties of the chondrocytes (Piperno *et al.* 1998), significantly reduced extracellular collagenase activity (Piperno *et al.* 2000) and significantly increased the rate of protein synthesis (Piperno *et al.* 2000). Osteoarthritic articular cartilage tissue samples harvested from rabbits that had been fed diets supplemented with glucosamine HCl (20 mg/kg body weight daily) exhibited significantly accelerated rates of synthesis of new proteoglycans compared to articular cartilage tissue samples harvested from unsupplemented animals (Oegema *et al.* 2002). Rotta Pharmaceuticals, Inc. also failed to present any evidence (scientific or anecdotal) to support their mistaken contention that "these other sources of glucosamine have not been shown through clinical trials to have the same effect on osteoarthritis as crystalline glucosamine sulfate." [pp. 2-3] Indeed, the human clinical trial (Braham *et al.* 2003) perhaps most relevant to the demonstration of the effectiveness of glucosamine compounds in the prevention of osteoarthritis was ignored in the Rotta petition. These investigators studied men and women aged 20 to 70 years (mean age: 43 years) who reported experiencing knee pain while participating in the normal activities of daily living "more often than not" although routine administration of anti-inflammatory or analgesic medications was not required. After random assignment to groups, subjects consumed either placebo or glucosamine HCl (2000 mg daily) for 12 weeks. While the means of previously-validated Knee Pain Scale scores and of a measure of knee-related quality of life improved with time in both groups, the improvements were significantly greater among subjects consuming glucosamine HCl. Not all measures of knee joint pain or function were affected to a significantly greater extent by glucosamine HCl; however, failure to observe significant differences in the magnitudes of improvement in those variables may well reflect the prediagnostic condition of the subjects and the 12-week duration of the study. As in all studies of glucosamine supplementation, there were no significant adverse reactions and the incidence or severity of inconsequential minor side effects were not affected by supplementation. The findings of these investigators are consistent with those of virtually all other investigators and confirm the biological activity of glucosamine HCl in the prevention of human osteoarthritis.

These findings also reinforce the argument documented in the Rotta petition (pp. 44-45) regarding the absolute necessity of recognizing the continuum from healthy tissues to compromised joint cartilage. Indeed, the entire preclinical and clinical literature demonstrates that the chondroprotective effects of glucosamine and chondroitin sulfate occur at the metabolic, biochemical, cellular and tissue levels where they inhibit cartilage degradation and stimulate production of new cartilage matrix. These chondroprotective effects of glucosamine, glucosamine sulfate and chondroitin sulfate are expressed both in

the absence of joint disease and in the presence of either asymptomatic clinically inapparent joint disease or clinically apparent joint disease. Therefore, the scientific evidence confirms that the physiological effects of glucosamine, glucosamine sulfate and chondroitin sulfate reflect the fundamental interactions of these dietary ingredients with the cells and matrix of hyaline articular cartilage, through which their chondroprotective effects are expressed.

The hypothesis put forward in the Rotta petition (p. 46) that the presence of sodium and chloride ions in the formulation is required for the biological activity of glucosamine sulfate also is contradicted by the available scientific literature. Specifically, the study by Thie *et al.* (2001) demonstrated that the active ingredients in formulations of glucosamine sulfate are either glucosamine, sulfate or glucosamine sulfate. In their 90-day study, these investigators observed a significantly greater decrease in pain among subjects with osteoarthritis of the temporomandibular joint following daily dietary supplementation with a glucosamine sulfate preparation that was not manufactured by Rotta Pharmaceuticals, Inc. (Jamieson™; Windsor, Ontario, Canada; 1500 mg daily) than was experienced by similar subjects following medication with ibuprofen (1200 mg daily). In addition, daily dietary supplementation with this formulation of glucosamine sulfate produced significant reduction in masticatory muscle pain and significant increases in pain-free mouth opening and in voluntary mouth opening; these improvements were of the same magnitudes as were those produced by ibuprofen medication. These findings confirm those emphasized in the Rotta petition (pp. 17-18; 20-21) demonstrating the comparability of the clinical effectiveness of glucosamine sulfate *per se* and that of ibuprofen and these results further confirm that the presence of sodium and chloride ions in the formulation is not necessary for the biological activity of glucosamine sulfate.

While the comments cited in the Rotta petition concerning the need to ensure product quality and composition (pp. 48-49) certainly were well-intentioned when made originally, the scientific literature demonstrates that continued allegiance to the fears of these commentors is not justified. The results reported by Thie *et al.* (2001) and Braham *et al.* (2003) document the effectiveness of glucosamine HCl and glucosamine sulfate *per se* in the prevention of human osteoarthritis and successfully refute the mistaken notion expressed by Rotta Pharmaceuticals, Inc. (pp. 3; 23; 25; 29; 30; 47-49; 52) that it may be necessary to "generalize" from the results of studies that focused on the products sold by Rotta Pharmaceuticals, Inc.

The argument put forth in the Rotta petition concerning the requirement for the inclusion of inorganic sulfate in any effective glucosamine formulation (pp. 7-8) is interesting. The petition does demonstrate that inorganic sulfate may well potentiate the biological activity of glucosamine in the prevention of human osteoarthritis. However, no evidence has been provided demonstrating that any such role is critical to or required for the biological activity of glucosamine *per se* in the prevention of human osteoarthritis. Furthermore, in assigning a critical role to inorganic sulfate ions, the Rotta petition suggests that it is these inorganic sulfate ions (and not the organic glucosamine molecule) that confers biological activity to glucosamine preparations. Similarly, this hypothesis is not supported by scientific evidence.

The disparaging comments within the Rotta petition concerning the effectiveness of chondroitin sulfate in the prevention of human osteoarthritis are at best confusing, particularly as those comments do not invalidate the findings of published human clinical trials and seem to reduce the credibility of the same meta-analyses upon which the Rotta petition has placed great emphasis in the consideration of glucosamine sulfate (pp. 10-11; 13-15). For example, Richy *et al.* (2003) concluded that daily dietary supplementation with chondroitin sulfate produced symptomatic efficacy "indistinguishable" from that of glucosamine sulfate and that "Chondroitin was found to be effective on Lesquesne Index, visual analog scale pain, mobility, and responding status." Similarly, McAlindon *et al.* (2000) concluded that dietary supplementation with chondroitin sulfates by individuals with osteoarthritis produced an approximately 50% reduction in pain with a similar improvement in function (a "large" effect consistently greater than that of placebo). These investigators did note that the quality of most published studies concerning dietary supplementation with chondroitin sulfates by individuals with osteoarthritis often has been poor and that the magnitude of the reported effects of dietary supplementation with chondroitin sulfates are likely to be inflated by weaknesses in the study designs and analyses. Nonetheless, it was concluded that the available published studies demonstrate a significant degree of efficacy for dietary supplementation with chondroitin sulfates. In addition, other meta-analysts also have evaluated the effectiveness of daily dietary supplementation with chondroitin sulfates. One group (Leeb *et al.* 2000) concluded that 7 randomized double-blind placebo-controlled studies (of adequate quality to include in their analysis) demonstrated that when consumed at 1200 mg daily for at least 120 days, dietary supplementation with chondroitin sulfates produced significantly greater reductions in the Lequesne Index of functional impairment and in the severity of pain (assessed using a visual analog scale) than did placebo (the effect size was "large"). In addition, 65% of subjects consuming chondroitin sulfates will be expected to benefit more than if they were consuming placebo and, in general, adverse effects were more frequent when placebo was consumed than when chondroitin sulfates were consumed. Other investigators concluded that dietary supplementation with chondroitin sulfates by individuals with osteoarthritis consistently produced significant decreases in joint pain and significant increases in joint function of small-to-moderate magnitude (Hauselmann 2001) and that dietary supplementation with chondroitin sulfates is "probably effective in osteoarthritis in reducing pain and in improving joint function" (Pendleton *et al.* 2000).

The application of a pharmacokinetic-pharmacodynamic model of intake-dependent effects resulted in the estimation that dietary supplementation with chondroitin sulfates "can reduce baseline pain and algofunctional indices by over 80%" (du Souich and Verges 2001). It was estimated that about half of this benefit can be experienced in about 35 days of supplementation.

In addition, there can be no doubt that dietary supplementation with chondroitin sulfate confers biological activity in the prevention of human osteoarthritis. Contrary to intimations by Rotta Pharmaceuticals, Inc. (p. 33) chondroitin sulfates are absorbed. In dogs, rats, mice and rabbits, about 0% to 15% of an ingested mix of chondroitin sulfates was absorbed intact (Dohlman 1956; Dziewiatkowski 1956; Konador and Kawiak 1976;

Andermann and Dietz 1982; Pamieri *et al.* 1990; Conte *et al.* 1995; Mobasheri *et al.* 2002). In these species, absorption favors chondroitin sulfate polymers with molecular weights <14,000 daltons (Palmieri *et al.* 1990). In all species studied, some inorganic $SO_4^{-2}$ also was absorbed following cleavage of $SO_4^{-2}$ from the chondroitin sulfate polymers by sulfatases (Dohlman 1956; Dziewiatkowski 1956; Konador and Kawiak 1976; Andermann and Dietz 1982; Pamieri *et al.* 1990; Conte *et al.* 1995; Mobasheri *et al.* 2002). In humans, between 0% and 15% of an oral bolus of chondroitin sulfates is absorbed intact into the blood (Morrison 1977; Baici *et al.* 1992; Murata, 1974; Conte *et al.* 1991). In addition, another 10% to 20% is absorbed following hydrolysis to smaller polymers (<5000 daltons) prior to absorption (Conte *et al.* 1991; Volpi 2002), although the biological activity of these smaller polymers has been questioned (Bucci 1995). The absorption of chondroitin sulfates probably is not nil; the consumption of either 800 mg or 3000 mg of mixed chondroitin sulfates significantly increased plasma chondroitin sulfate concentration 3 hours after ingestion (Gross 1983; Ronca *et al.* 1998) and the consumption of 800 mg daily for 5 days increased plasma chondroitin sulfate concentration from undetectable levels to a mean of 1.80 mcg/mL, suggesting that the systemic bioavailability of intact chondroitin sulfates in humans is about 12% of the amount ingested (Ronca *et al.* 1998).

Statements in the Rotta petition that the effectiveness of combinations of glucosamine HCl and chondroitin sulfate is "anecdotal" (p. 33) and that "there is no scientific proof for this claim" (p. 33) are contradicted by the evidence obtained in randomized placebo-controlled clinical trials (Leffler *et al.* 1999; Das and Hammad 2000) provided on page 32 of the petition, as well as by additional evidence from another randomized placebo-controlled clinical trial (Nguyen *et al.* 2001) and an open-label study (Shankland 1998). Furthermore, in its discussion of these trials the Rotta petition developed an inconsistency, simultaneously claiming that the effectiveness in the prevention of human osteoarthritis of daily dietary supplementation with chondroitin sulfate "may be similar to that of low dose glucosamine sulfate;" (p. 33) "other sources of glucosamine have not been shown through clinical trials to have the same effect on osteoarthritis as crystalline glucosamine sulfate;" (p. 3) it is not possible "to distinguish between the effects of glucosamine [HCl] alone or of the combination [of glucosamine HCl plus chondroitin sulfate];" (p. 33) and the trials presented on page 32 are considered by Rotta Pharmaceuticals, Inc. to be "favorable studies" (p. 32). Logically, if chondroitin sulfate is ineffective, then the 4 studies cited that describe the results of supplementation with glucosamine HCl and chondroitin sulfate demonstrate the effectiveness of glucosamine HCl. Similarly, if glucosamine HCl is ineffective, then the 4 studies cited that describe the results of supplementation with glucosamine HCl and chondroitin sulfate demonstrate the effectiveness of chondroitin sulfate. On the other hand, the conclusion stated in the Rotta petition that it is not possible "to distinguish between the effects of glucosamine [HCl] alone or of the combination [of glucosamine HCl plus chondroitin sulfate]" (p. 33) supports the conclusion that glucosamine and chondroitin sulfate may reduce the risks of osteoarthritis, osteoarthritis-related joint pain, tenderness and swelling, joint degeneration and cartilage deterioration.

In its invocation of the FDA decision concerning soluble dietary fiber (62 *Fed Reg* 3583, 3587-3588, Jan 23, 1997), the Rotta petition states "health claim eligibility must be restricted to the specific substances for which the claimed health benefit has been demonstrated by credible scientific evidence" (p. 49). As documented within the Rotta petition and above, we agree that this requirement is satisfied by glucosamine HCl, by chondroitin sulfate and by their combination.

Finally, because glucosamine sulfate preparations merely act as carriers of glucosamine (the active component) and sulfate, the health claims proposed by Rotta Pharmaceuticals, Inc. would most correctly be limited to "glucosamine" rather than its carrier form (glucosamine sulfate). However, because gastrointestinal processing of glucosamine sulfate converts it into active glucosamine, it would be appropriate to include "glucosamine sulfate" in health claims that educate the public concerning the biological activity of glucosamine in the prevention of human osteoarthritis.

June 3, 2004

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[1] Dr. Glade's signature for this report is on file with Emord & Associates. It is available upon request.

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